

PATENT APPLICATION

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicants: Perry A. Frey *et al.*

Title: DNA Molecules Encoding Bacterial  
Lysine 2,3-Aminomutase

Appl. No.: 09/847,010

Filing Date: 05/01/2001

Examiner: Richard G. Hutson

Art Unit: 1652

**DECLARATION UNDER 37 C.F.R. §1.132 OF DR. VICTORIA SUTTON**

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Dear Examiner:

I, Dr. Victoria Sutton, state and declare that:

1. In 1997 I received my Bachelor of Science degree in Biology from Western Michigan University. I received my Ph.D. from the University of Wisconsin - Madison in Cellular and Molecular Biology in 2004 . During my doctoral research, I performed numerous sequence alignments.
2. I am a temporary employee of the Wisconsin Alumni Research Foundation.
3. I performed a sequence alignment of the lysine 2,3-aminomutase polypeptide sequences disclosed in U.S. Patent Application No. 09/847,010 in conjunction with Eric Cabot using the BLOSUM62 scoring matrix as implemented in ClustalW, using the program's default parameters. (See Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Research, 22:4673-4680.) These algorithms or similar ones were publicly available on or before May 1, 2001, the filing date for U.S. Patent Application No. 09/847,010. The counterpart amino acid of each sequence was compared at each position of SEQ ID NO: 2, the clostridial lysine 2,3-aminomutase.

4. The resulting alignment of the sequences at each position of the clostridial lysine 2,3-aminomutase enzyme is shown in the Appendix. The first column lists each amino acid position in the clostridial lysine 2,3-aminomutase enzyme, and the second column lists the clostridial amino acid at that position. The third column shows each counterpart amino acid in the seven other lysine 2,3-aminomutase sequences for each clostridial position; dashes indicate the absence of a counterpart amino acid. The final column shows the percent sequence identity among all eight sequences.
5. Based on percent sequence identity, the alignment shows which positions are likely to be tolerant or intolerant of amino acid substitutions. Thus, for example, positions 48-52, 54-58, 87-95, 140-152, 215-217, 267-270, and 373-382 have low sequence identity (50% or less), indicating a likely tolerance for amino acid substitutions. By contrast, residues 124, 128, 129, 131, and 133 are all 100% conserved and are unlikely to tolerate even a conservative amino acid substitution well.
6. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent resulting therefrom.

Date: February 23, 2007



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Dr. Victoria R. Sutton

## APPENDIX

SB4		Amino acids of other lysine 2,3-aminomutases	% Sequence Identity
Position	SB4 Acid	SB4 Acid	
1	M	--AL---	33.33
2	I	--EK---	33.33
3	N	--SN---	66.67
4	R	--RK---	66.67
5	R	--RW---	66.67
6	Y	--KY---	66.67
7	E	IIYE-R-	33.33
8	L	VLYL-F-	50.00
9	F	TPFWWF-	42.86
10	K	LQPKQE-	28.57
11	D	NEDDGNA-	42.86
12	V	TPVVVVV	75.00
13	S	PVTPPPS	25.00
14	D	SIDEDEM	37.50
15	A	RREEENA	25.00
16	D	EEQKQLE	12.50
17	W	DEWWWWWC	62.50
18	N	-QNNYRT	42.86
19	D	-NDDDSR	57.14
20	W	WWWWWYE	75.00
21	R	LLHLKEQ	12.50
22	W	TTWWWWR	62.50
23	Q	QIQQQQK	75.00
24	V	LLVLLIR	25.00
25	R	AKLTQQR	25.00
26	N	DNNHNNG	62.50
27	R	VARTRRA	50.00
28	I	VIIVIIG	62.50
29	E	TSERNKR	25.00
30	T	DDTTSTA	50.00
31	V	PPLLVLD	25.00
32	E	DKDDEKE	37.50
33	E	ELQDEEH	50.00
34	L	LLLLLIW	75.00
35	K	LLKKQKR	50.00
36	K	RKKKEKT	62.50
37	Y	LAYVYVL	37.50
38	I	LLVILLS	25.00
39	P	NNTNTKP	25.00
40	L	ILLLLA	75.00
41	T	DPTTTLS	50.00
42	K	EEAEEP-	14.29
43	E	EDEDSE-	57.14
44	E	KDEEEE-	71.43
45	E	LFEEYE-	57.14
46	E	LEEERE-	71.43
47	G	AQGGGG-	71.43
48	V	GSVVAI-	42.86

49	A	RIKRSK-	14.29
50	Q	SAEIARC	12.50
51	C	AASSETA	12.50
52	V	KRLTGQA	12.50
53	K	KKKK-GD	71.43
54	S	LLVTILA	12.50
55	L	FFLIFYL	37.50
56	R	ASRPRPT	37.50
57	M	LLMLLF	25.00
58	A	RRANDAH	37.50
59	I	VVIIIII	75.00
60	T	PPTTTTS	62.50
61	P	RQPPPPP	75.00
62	Y	SPYYYYA	62.50
63	Y	FFYYFYY	62.50
64	L	IILAALA	37.50
65	S	DDSSSSH	62.50
66	L	RKLLLLL	75.00
67	I	MIIMMII	62.50
68	D	EEDDDNA	50.00
69	P	KKPPPPQ	62.50
70	N	GGEDEEA	12.50
71	D	NNNNNDQ	37.50
72	P	PPPPPPD	87.50
73	N	DQNRTKA	25.00
74	D	DDCCCDQ	50.00
75	P	PPPPPPA	87.50
76	V	LLIVVIL	37.50
77	R	LFRRRRK	62.50
78	K	RLKMRLR	25.00
79	Q	QQQQQQQ	100.00
80	A	VVASVAV	37.50
81	I	LMIVIIC	50.00
82	P	TCPPPPF	62.50
83	T	SSTLTRA	37.50
84	A	QDHSEVP	12.50
85	L	DLQEEVQ	25.00
86	E	EEEEEEE	100.00
87	L	FFLMLVR	37.50
88	N	VVHQDV	12.50
89	K	IQRKPEV	25.00
90	A	AAATFKH	50.00
91	A	PEPKTVA	25.00
92	A	GGEYSQC	12.50
93	D	FFDDDMSE	37.50
94	L	SSQLMAC	25.00
95	E	TTVEEGA	37.50
96	D	DDDDDED	87.50
97	P	PPPPSPP	87.50
98	L	LLLLLDL	87.50
99	H	EESHAAG	25.00
100	E	EEEELE	87.50

101	D	QKDDDKD	62.50
102	T	HNEEKER	12.50
103	D	SADDHEY	37.50
104	P	VAPPDV	50.00
105	V	VVVVVIT	75.00
106	P	PPPPPPP	100.00
107	G	GNGGGGF	75.00
108	L	LILLLLL	87.50
109	T	LLTTVTV	50.00
110	H	HHHHHHH	100.00
111	R	KKRRRRQ	62.50
112	Y	YYYYYYY	100.00
113	P	HRPPPPA	62.50
114	D	NNDDDDN	62.50
115	R	RRRRRRR	100.00
116	V	ALVVVVV	75.00
117	L	LLLLLL	100.00
118	L	LFFFMLM	37.50
119	L	LMLLLNL	75.00
120	I	VAIVVVA	25.00
121	T	KKTTTTT	75.00
122	D	GGDNTTG	25.00
123	M	GGKQQFR	12.50
124	C	CCCCCCC	100.00
125	S	AASSAAF	37.50
126	M	VVMMMSVS	37.50
127	Y	NNYYYYH	62.50
128	C	CCCCCC	100.00
129	R	RRRRRRR	100.00
130	H	YYHYHY	37.50
131	C	CCCCCC	100.00
132	T	FFTTTMF	50.00
133	R	RRRRRRR	100.00
134	R	RRRRSKR	75.00
135	R	HHRRRRG	62.50
136	F	FFFFIIF	75.00
137	A	PPASVF	25.00
138	G	YYGGGSA	50.00
139	Q	ADQQDQQ	62.50
140	S	EEKIPGR	12.50
141	D	NNDGTEA	25.00
142	D	QPAMERG	12.50
143	S	GGSGTAW	25.00
144	M	-SVFRI	16.67
145	P	NNPPNTP	50.00
146	M	KKSXPKN	12.50
147	E	RKEKAEE	50.00
148	R	NSRQEEE	25.00
149	I	WWILYIR	37.50
150	D	QQDDEDE	50.00
151	K	TLRAATK	25.00
152	A	AACAQMI	50.00

153	I	LLIILII	62.50
154	D	EDDANDT	50.00
155	Y	YYYYYYY	100.00
156	I	VIIILIL	62.50
157	R	AAARRKR	50.00
158	N	AANENRA	37.50
159	T	HHTTTHT	62.50
160	P	PSPPPPEP	75.00
161	Q	EETEQES	25.00
162	V	LIVIVIV	50.00
163	R	DERRRK	62.50
164	D	EEDDDDE	62.50
165	V	MVVCVVI	62.50
166	L	IILLLL	75.00
167	L	FFLILIV	37.50
168	S	SSSSSSS	100.00
169	G	GGGGGGG	100.00
170	G	GGGGGGG	100.00
171	D	DDDDDED	87.50
172	A	PPAGPPP	25.00
173	L	LLLLLL	100.00
174	L	MMMLTST	37.50
175	V	AAVILLG	25.00
176	S	KKSNASS	50.00
177	D	DDDDPLF	62.50
178	E	HHEQKEA	37.50
179	T	EERIVKQ	12.50
180	L	LLLLLV	87.50
181	E	DAEEGET	50.00
182	Y	WWYYRYS	50.00
183	I	LLIILL	37.50
184	I	LILLLF	25.00
185	A	TKKKSSR	12.50
186	K	QHREERA	12.50
187	L	LLLLLL	100.00
188	R	EERRRRR	75.00
189	E	ANESKES	37.50
190	I	IIIIIV	87.50
191	P	PPPPEKA	62.50
192	H	HHHHHHHP	87.50
193	V	ILVLIVD	37.50
194	E	KQEEEL	62.50
195	I	RRIVIII	62.50
196	V	LLVIII	25.00
197	R	RRRRRRR	100.00
198	I	IIIIFL	75.00
199	G	HHGGGGC	62.50
200	S	STSTTTT	37.50
201	R	RRRRRRR	100.00
202	T	LLTAVLA	25.00
203	P	PPPPPPV	87.50
204	V	IVVVVT	75.00

205	V	VVVVFLF	62.50
206	L	IILFMAA	25.00
207	P	PPPPPPP	100.00
208	Q	AQQQMQQ	75.00
209	R	RRRRRRA	87.50
210	I	IIIVFF	62.50
211	T	TTTTTFT	87.50
212	P	AEPDQDP	37.50
213	E	LFQHEKE	37.50
214	L	VCLLLL	75.00
215	V	ETVCCLI	25.00
216	N	CLDEDAA	12.50
217	M	FLMITIF	25.00
218	L	AALLLLL	75.00
219	K	REKKAEQ	37.50
220	K	STKKEKE	50.00
221	Y	TRYHHYM	50.00
222	H	LLHHHSK	50.00
223	P	QQPPPPP	75.00
224	V	ITVVLIV	50.00
225	W	LVWWWWW	75.00
226	L	LMLLMII	50.00
227	N	VVN>NNNI	62.50
228	T	NTTTITP	62.50
229	H	HHHHHHH	100.00
230	F	IIFVF	50.00
231	N	NNNNNNN	100.00
232	H	HHHTHHH	87.50
233	P	APPSPPP	75.00
234	N	NNNIKNA	62.50
235	E	EEEEEEE	100.00
236	I	VIVMIL	50.00
237	T	DDTTTG	62.50
238	E	EQEEPES	62.50
239	E	TIEEYT	50.00
240	S	FFASVAQ	25.00
241	T	RAVVAER	12.50
242	R	QHEEEEA	12.50
243	A	AAAAAAV	87.50
244	C	MMCCCVL	50.00
245	Q	AQEEDDE	25.00
246	L	KKRKRRA	12.50
247	L	LLMLLLC	75.00
248	A	RNAVTLV	25.00
249	D	RANNRG	12.50
250	A	VVAAARA	62.50
251	G	GNGGGGG	87.50
252	V	VVIVVIL	62.50
253	P	TPPPPPP	75.00
254	L	LLLVLVV	62.50
255	G	LLGGGNQ	50.00
256	N	NNNNNNNS	87.50

257	Q	QQQQQQQ	100.00
258	S	SSTASTS	62.50
259	V	VVVVVVV	100.00
260	L	LLLVLLL	87.50
261	L	LLLLLLL	100.00
262	R	RKRARKR	62.50
263	G	DGGGGGG	87.50
264	V	VVIVVV	75.00
265	N	NNNNNNN	100.00
266	D	DDDDDDD	100.00
267	C	NDCSHDS	25.00
268	V	AATVPV	37.50
269	H	QQHPVEE	25.00
270	V	TIVIIVT	37.50
271	M	LLMMMLL	62.50
272	K	AKKKQLC	50.00
273	E	NIRKKKT	12.50
274	L	LLLLLLL	100.00
275	V	SSVMLFF	25.00
276	N	NDHHRRH	25.00
277	K	AKLDEKA	37.50
278	L	LLLLLLL	100.00
279	V	FFVVVLT	50.00
280	K	DQKKKRC	50.00
281	I	ATMIIIL	50.00
282	R	GGRRRKG	50.00
283	V	VIVVVVV	87.50
284	R	MLRRRK	50.00
285	P	PPPPPPP	100.00
286	Y	YYYYYYQG	75.00
287	Y	YYYYYYY	100.00
288	I	LLIIILL	50.00
289	Y	HHYYFF	50.00
290	Q	VLVQQHQ	50.00
291	C	LLCCCCL	62.50
292	D	DDDDDDD	100.00
293	L	KKLLLPL	62.50
294	S	VVSSVIA	37.50
295	L	QQLEHKP	25.00
296	G	GGGGGGG	100.00
297	L	AAIIAAT	12.50
298	E	ASGGVG	12.50
299	H	HHHHHHHD	87.50
300	F	FFFFLFF	87.50
301	R	MLRRRRR	75.00
302	T	VITATT	50.00
303	P	SSPPTTP	50.00
304	V	DDVVVIL	50.00
305	S	DISSSDS	62.50
306	K	EEKKKKD	62.50
307	G	AAGGGGT	62.50
308	I	RMILLLL	25.00

309	E	QQEEEEA	62.50
310	I	IIIIIL	87.50
311	I	MYIIMMW	37.50
312	E	RKEEERR	50.00
313	G	ETNGSYT	25.00
314	L	LLLLLLL	100.00
315	R	LQRRRRK	62.50
316	G	TSGGGGE	62.50
317	H	LLHHHRR	50.00
318	T	VTTTLL	62.50
319	S	SSSSSSS	100.00
320	G	GGGGGGG	100.00
321	Y	YYYYYFL	75.00
322	C	LLAASGS	12.50
323	V	VVVVVL	75.00
324	P	PPPPPPP	100.00
325	T	KKTTTTT	75.00
326	F	LLFFYYL	37.50
327	V	AAVVVAA	50.00
328	V	RRVVVVV	75.00
329	D	EEDDDDD	75.00
330	A	IIAALL	50.00
331	P	GAPPPPP	75.00
332	G	GGGGGGG	100.00
333	G	EEGGGGG	75.00
334	G	PPGGKG	62.50
335	G	SNGGGGG	75.00
336	K	KKKKKKK	100.00
337	T	TTIIIF	37.50
338	P	P-PAPPP	85.71
339	V	L-LVLL	42.86
340	M	D-MQALV	28.57
341	P	L-PPPPA	71.43
342	N	Q-NNNNL	71.43
343	Y	LLYYYYA	62.50
344	V	RYVVVVL	62.50
345	I	QAVLLKQ	12.50
346	S	QESSSKQ	50.00
347	Q	-QQHRD	50.00
348	S	-SSSKV	66.67
349	H	-PPPGT	16.67
350	D	-RDENW	33.33
351	K	-HKKKH	66.67
352	V	-VVLFQ	50.00
353	I	-VIIWE	50.00
354	L	-LLLFR	66.67
355	R	-RRREE	66.67
356	N	-NNNSA	66.67
357	F	-YFFFF	83.33
358	E	--EEETS	66.67
359	G	--GGGGA	83.33
360	V	--VYER	50.00

361	I	--IIIVG	66.67
362	T	--TTAVI	50.00
363	T	--TSAED	33.33
364	Y	--YYYYG	83.33
365	S	--TPSEA	33.33
366	E	--EEEVW	66.67
367	P	--PPPTY	66.67
368	I	--EETET	16.67
369	N	--NNDVY	50.00
370	Y	--YYWP	66.67
371	T	--HITEF	33.33
372	P	--EPGP-	60.00
373	G	--EED--	25.00
374	C	--CSM--	50.00
375	N	--DVA--	25.00
376	C	--CFI--	50.00
377	D	--EPP--	25.00
378	V	--DED--	25.00
379	C	--CTD--	50.00
380	T	--RAW--	25.00
381	G	--ADI--	25.00
382	K	--GKR--	50.00
383	K	--KKK--	100.00
384	K	---E--	50.00
385	V	---P--	50.00
386	H	--HEG--	50.00
387	K	--KPO--	50.00
388	V	--EIT--	25.00
389	G	--GGG--	100.00
390	V	--VLI--	50.00
391	A	--ASF--	50.00
392	G	--AAG--	50.00
393	L	--LIL--	75.00
394	L	--SFM--	25.00
395	N	--GAE--	25.00
396	G	--GDG--	75.00
397	E	--QKE--	50.00
398	G	--QER--	25.00
399	M	--LVI--	25.00
400	A	--ASS--	50.00
401	L	--IFI--	25.00
402	E	--ETE--	75.00
403	P	--PPP--	100.00
404	V	--SE--	33.33
405	G	--DN--	33.33
406	L	--LV--	66.67
407	E	--AD--	33.33
408	R	--RR--	100.00
409	N	--KI--	33.33
410	K	--KK--	100.00
411	R	--RR--	100.00
412	H	--KQ--	33.33

413	V	--FL---	33.33
414	Q	--DK--	33.33
415	E	--KE--	66.67